overlapping with the Vitiligo Impact Patient scale. As both scales were tested on geographically diverse populations, this supports the validity of the approach. Previously, it has been shown that several items of the dermatology life quality index and Skindex are subject to bias according to cultural differences (Nijsten et al., 2007), suggesting that for vitiligo, a global consensus might best determine the impact score that should be employed in epidemiological studies and in clinical trials. Validation in an international multicenter study would likely be required.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by a postdoctoral research grant to RS from the Ghent University Special Research Fund (BOF; grant number: 01P12914) and a research grant to NvG from the Scientific Research Foundation-Flanders (FWO Senior Clinical Investigator; grant number: FWO11/FKM/001).

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An Unexpected Role for TRPV4 in Serotonin-Mediated Itch

Lindsey M. Snyder[1,2], Marissa S. Kuzirian[1,2] and Sarah E. Ross[1,2,3]

Previous studies have revealed that TRPV1 and TRPA1 function downstream of many itch receptors, where they mediate inward current to trigger action potentials in primary afferents. Although other TRP channels, such as TRPV4, are expressed in primary afferents, whether or not they play an analogous role in itch was previously unknown. Now, Akiyama et al. provide evidence that TRPV4 is a key mediator of serotonin-induced itch. This finding is important because it uncovers an unanticipated role for TRPV4 in itch, thereby identifying a novel therapeutic target.

Journal of Investigative Dermatology (2016) **136,** 7–9. doi:10.1016/j.jid.2015.11.010

Clinical relevance of itch

Chronic itch, which is defined as itch lasting more than 6 weeks, is a prevalent problem that occurs in approximately 10% of the population (Mollanazar et al., 2015). Chronic itch conditions negatively affect quality of life, and yet there are no therapies that are both efficacious and selective for itch. The lack of effective treatment is partly attributable to a poor understanding of the mechanisms that underlie it. Although antihistamines are frequently prescribed as a treatment for itch, they are typically ineffective because most types of chronic itch are not histamine-mediated (Mollanazar et al., 2015). Unfortunately, although there are numerous mediators that can cause itch, the factors that are responsible in most circumstances of chronic itch are largely unknown. One candidate mediator is serotonin (5-hydroxytryptamine, 5-HT). Human psychophysical studies have shown that the application of serotonin into the skin causes itch (Weisshaar et al., 2004). In rodents, serotonin is a key component of mast cells, and it is a potent mediator of itch. However, until recently, the mechanisms through which serotonin causes itch have remained uncertain.

TRPs as downstream mediators of itch (pruritogens)

Many pruritogens bind to metabotropic receptors on primary sensory neurons; however, these receptors must be coupled to ionotropic channels via intracellular signaling pathways to allow sufficient current influx to generate action potentials. Several groups have shown that the cation channels TRPV1 and TRPA1 are coupled to different pruritogen receptors and that they are critical for different forms of itch transmission (Ross, 2011). More specifically, TRPV1 is required for histaminergic itch, whereas TRPA1 is required for several types of nonhistaminergic itch, such as that induced by chloroquine, BAM8-22, IL-31, endothelin-1, thymic stromal lymphopoietin, and bile acids. Until recently, whether serotonin receptors were likewise coupled to TRPs remained unknown.

> TRPV4 is a key mediator of serotonin-induced itch, thereby identifying a novel therapeutic target.

Mechanisms of serotonin-induced itch

Understanding serotonin-mediated itch has been complicated by the fact that there are numerous serotonin receptors that are expressed on primary afferents, as well as on immune mediators that

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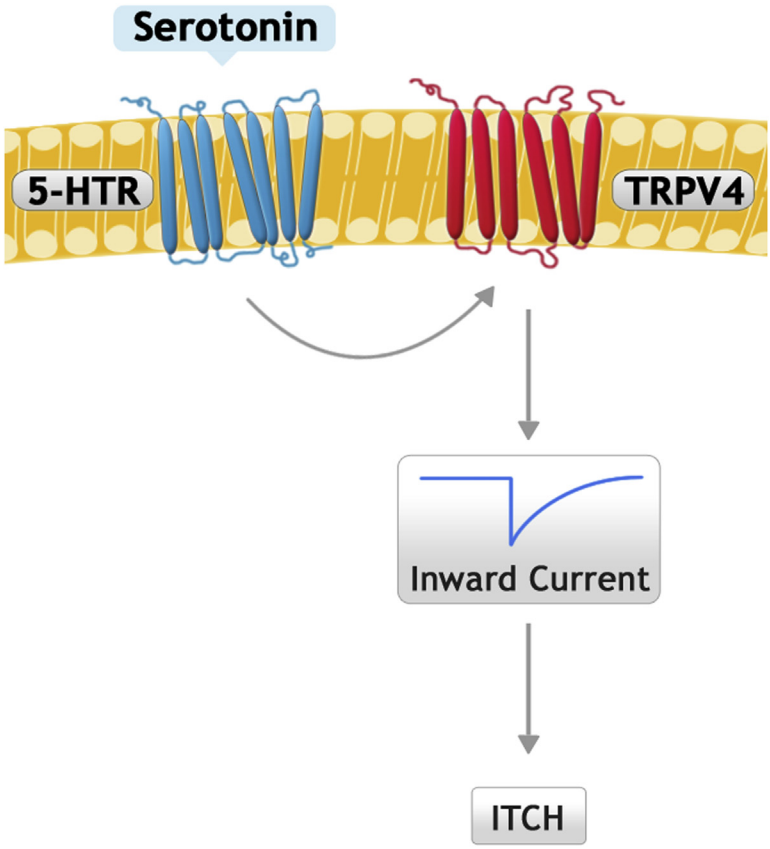

Figure 1. TRPV4 is a key mediator of serotonin-induced itch. Akiyama et al. (2016) provide evidence that the serotonin receptor (5-HTR, blue) couples to TRPV4 (red) to mediate the activation of primary sensory afferents, which triggers itch.

could be involved in itch. It was previously hypothesized that the primary pathway through which serotonin causes itch is via stimulation of histamine release from mast cells. However, contrary to this idea, antihistamines failed to reduce serotonin-induced itch sensation in humans (Hosogi et al., 2006). Thus, the mechanisms of serotonin-induced itch remained unknown.

A role for 5-HT$_7$ and TRPA1 in serotonin-mediated itch

A recent study has demonstrated that one way in which serotonin induces itch is via direct activation of 5-HT$_7$ (encoded by *HTR7*), which is expressed on subsets of primary sensory afferents (Morita et al., 2015). In this study, mice lacking either *HTR7* or *TRPA1* showed substantially reduced scratching behavior in response to an intradermal injection of a 5-HT$_7$-selective agonist. Furthermore, *HTR7* and *TRPA1* knockout mice scratched considerably less in a model of atopic dermatitis. However, it seemed likely that this was only part of the serotonin-itch story, because the 5-HT$_2$-selective agonist, α-methyl-5HT, is a potent pruritogen in mice. Akiyama et al. (2016) provide further insight into the molecular players involved in serotonin-evoked itch by defining a TRPV4-dependent pathway that is likely to be downstream of 5-HT$_2$-mediated itch.

An unexpected role for TRPV4 in serotonin-mediated itch

The original goal of this study was to investigate a possible role for TRPV4 in itch. TRPV4 is upregulated in the skin of individuals with certain itch conditions (Moore et al., 2013; Yang et al., 2015), suggesting that it may be involved in itch in humans. Interestingly, *TRPV4* knockout mice displayed a significant reduction in scratching behavior in response to serotonin, but not to histamine, chloroquine, or SLIGRL (Akiyama et al., 2016). A TRPV4 antagonist also reduced substantially the amount of serotonin-evoked scratching, supporting the idea that TRPV4 is critical to serotonin signaling in normal mice. Importantly, the authors showed that the change in response to serotonin in the *TRPV4* knockout mice was specifically a decrease in serotonin-evoked itch behaviors, and not a change in serotonin-evoked pain behaviors. This study demonstrates that TRPV4 is a key downstream component of serotonin-evoked itch (Figure 1).

To link serotonin to TRPV4 and the activation of sensory neurons, the authors visualized calcium responses to serotonin in dorsal root ganglion neurons. They found that approximately 90% of sensory neurons that respond to serotonin also expressed TRPV4. Serotonin-mediated activation was dependent on TRPV4, as a TRPV4 antagonist reduced significantly the calcium response to the application of serotonin. In support of this finding, the authors demonstrated that the proportion of neurons that responded to serotonin was reduced significantly in *TRPV4* knockout mice. Interestingly, the proportion of neurons responding to other types of pruritogens did not change in mice lacking *TRPV4*, indicating that TRPV4 plays an important and specific role in responses to serotonin in primary sensory neurons.

To identify the receptor through which serotonin acts, Akiyama et al. (2016) used subtype-specific antagonists for 5-HT$_1$ and 5-HT$_2$. The 5-HT$_2$ antagonist, but not the 5-HT$_1$ antagonist, reduced serotonin-evoked scratching. This finding raises the possibility that 5-HT$_2$, acting via TRPV4, is a key mediator of serotonin-evoked itch. Thus, there appear to be at least two distinct pathways through which serotonin mediates itch: a TRPA1-dependent pathway that mediates 5-HT$_7$-mediated itch, as well as a TRPV4-dependent pathway that likely mediates 5-HT$_2$-mediated itch. What remains to be tested is whether these receptors are expressed on distinct or overlapping populations of primary sensory afferents.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by NIH grants R01 AR063772 and R21 AR064445 to SER. LMS and MSK are supported by a T32 NS 73548-3.

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TIGIT-CD155 Interactions in Melanoma: A Novel Co-Inhibitory Pathway with Potential for Clinical Intervention

Karsten Mahnke[1] and Alexander H. Enk[1]

Inozume et al. describe a novel immunosuppressive mechanism in melanoma that is triggered by the interaction between CD155 (expressed by melanomas) and T-cell Ig and ITIM domain (TIGIT) (expressed by tumor infiltrating lymphocytes). This pathway exists in addition to the "classical" co-inhibitory PD-1-PD-L1 pathway. Hence, the combinatorial blockage of both pathways by specific antibodies resulted in the greatly enhanced effector function of melanoma-specific cytotoxic T cells. Given that CD155-TIGIT signaling exerts potent inhibitory action in different subsets of immune cells and that CD155 is expressed broadly in several tumor entities, this report points toward a novel and promising therapeutic strategy to combine different checkpoint blocking agents for greater success in antitumor therapy.

Journal of Investigative Dermatology (2016) **136,** 9–11. doi:10.1016/j.jid.2015.10.048

The inhibitory TIGIT-CD155 pathway

Immune responses are regulated by the concerted action of immunostimulatory and immunosuppressive signals that are conveyed between antigen-presenting cells and effector T cells. The balance of this interplay is often tipped by tumors toward a more immunosuppressive environment. Thereby, tumors may utilize receptors or ligands that normally would trigger inhibitory pathways in effector T cells. Several receptors and/or ligands, such as PD-L1, PD-1, CTLA-4, CD155, and TIGIT, have been identified as important regulatory molecules. Among these the most recently identified inhibitory pair is T-cell Ig and ITIM domain (TIGIT) and its ligand CD155 (Yu et al., 2009).

The molecule TIGIT was identified initially in a genome-wide screen for molecules that (a) are expressed by immune cells and (b) contain the well-defined inhibitory ITIM motif, which is known to mediate inactivating signals in a variety of immune cells. TIGIT is expressed normally by activated T cells, regulatory T cells (Treg), and natural killer (NK) cells. The poliovirus receptor (CD155) and Nectin-2 (CD112) have been identified as relevant ligands (Li et al., 2014; Stanietsky et al., 2009; Yu et al., 2009). TIGIT competes with the molecules CD226 and CD96 for binding to CD155 and CD112, respectively, but among all respective receptor-ligand combinations, TIGIT exhibits the strongest affinity for CD155. Interestingly, the other known receptor for CD155, CD226, conveys activating signals into T cells, whereas TIGIT clearly has suppressive activity. This competition of an inhibitory receptor (TIGIT) and a stimulatory receptor (CD226) for one ligand (CD155) is reminiscent of the CTLA4-CD28-B7 axis. This similarity of the CD155-TIGIT interaction to the already known suppressive CTLA4-CD28 pathway, as well as the expression of TIGIT by exhausted T cells and by Treg, prompted investigators to speculate that the TIGIT-CD155 axis in tumors might serve as a checkpoint for tumor growth (Chauvin et al., 2015; Johnston et al., 2015; Li et al., 2014).

Inozume et al. (2016) report that melanoma cell lines, as well as melanoma tissue samples, express CD155 strongly. In functional assays, the authors show that the effector functions are suppressed completely in tumor-specific T cells that express the corresponding inhibitory receptor TIGIT. But antibody-mediated blockade of CD155 could, at least partially, reverse these effects, leading to increased antitumor activity by cytotoxic T cells against the melanoma cells. Interestingly, simultaneous blockade of the previously identified checkpoint molecule PD-L1 had an additive effect, with anti-PD-L1 and anti-CD155 antibodies together restoring the effector function of tumor infiltrating lymphocytes almost completely. Thus, in addition to the already defined PD-L1-PD-1 axis in melanomas, a second inhibitory pathway, characterized by CD155-TIGIT interplay, is active.

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